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**Short Term Precision Error of Body Composition Assessment Methods in
Resistance Trained Male Athletes**

Ava D Farley¹, Gary J Slater¹, Karen Hind²

¹School of Health and Sport Sciences, University of the Sunshine Coast, Sippy Downs, 4556,
Queensland, Australia (ADF, GJS)

²Department of Sport and Exercise Sciences, Durham University, Durham, DH1 3HN, United
Kingdom (KH)

Address for correspondence:

Ava Farley

School of Health and Sport Sciences

University of the Sunshine Coast

Maroochydore DC 4558

Queensland, Australia

P: +61 7 5459 4605

Abstract

Athletic populations require high precision body composition assessment to identify true change. Least Significant Change (LSC) determines technical error via same-day consecutive tests but doesn't integrate biological variation, being more relevant for longitudinal monitoring. The aim of this study was to assess biological variation using LSC measures from body composition methods used on athletes, including surface anthropometry (SA), air displacement plethysmography (BOD POD), dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance spectroscopy (BIS). Thirty-two athletic males (age: 31 ± 7 yr; stature: 183 ± 7 cm; mass 92 ± 10 kg) underwent three testing sessions over two days using four methods. LSC values were calculated from differences in Day¹Test¹ vs Day¹Test² (same-day precision), as well as Day¹Test¹ vs Day² (consecutive-day precision). There was high agreement between same-day and consecutive-day FM and FFM measurements for all methods. Consecutive-day precision error (PE) in comparison to same-day, was 50% higher for FM estimates from BIS (3607g vs 2331g), 25% higher from BODPOD (1943g vs 1448g) and DXA (1615g vs 1204g), but negligible from SA (442g vs 586g). Consecutive-day PE for FFM was 50% higher from BIS (3966g vs 2276g) and SA (1159g vs 568g), and 25% higher from BODPOD (1894g vs 1450g) and DXA (1967g vs 1461g) than same-day. PE in consecutive-day analysis considers both technical error and biological variation, enhancing identification of small, yet significant changes in body composition of resistance trained male athletes. Given change in physique is likely to be small in this population, the use of DXA, BOD POD or SA is recommended.

Key Words: Body composition, least significant change, BOD POD, dual energy x-ray absorptiometry, bioelectrical impedance spectroscopy, anthropometry, fat mass, fat free mass.

Introduction

The association between athletic physique traits and competitive sporting success is well established (Meyer et al., 2013; Olds, 2001). In sports requiring high force production, athletes with high levels of muscularity can gain a competitive advantage (Bilsborough, Greenway, Livingston, Cordy, & Coutts, 2016; Gabbett, 2009; Olds, 2001) yet these athletes tend to see only small adaptations or improvements in physique over time (Binkley, Daughters, Weidauer, & Vukovich, 2015; Harley, Hind, & O'Hara, 2011; Lees et al., 2017; Smart, Hopkins, & Gill, 2013). Given this, assessment methods with high precision are required to measure body composition on a regular basis in these athletes. By accurately quantifying changes in physique, more refined training and dietary interventions may be implemented which can positively influence performance outcomes (Slater et al., 2005).

A variety of body composition assessment methods are available to quantify fat free mass (FFM) and fat mass (FM) (Ackland et al., 2012; Kerr, Slater, & Byrne, 2017). Depending on time and resources, the four most popular methods used on athletic populations are air displacement plethysmography (BODPOD), dual energy x-ray absorptiometry (DXA), bioelectrical impedance spectroscopy (BIS) and surface anthropometry (SA) (Meyer et al., 2013). Despite differences in technology, resources and technical expertise required, they are all susceptible to technical error and biological variation (Ackland et al., 2012; Meyer et al., 2013), significantly affecting precision (Kerr et al., 2017; Kerr, Slater, & Byrne, 2018). Technical error is influenced by quality control procedures such as subject clothing (D. A. Fields, Hunter, & Goran, 2000; Vescovi, Zimmerman, Miller, & Fernhall, 2002), and positioning during assessment (Kerr, Slater, Byrne, & Nana, 2016; Lambrinoudaki et al., 1998; Tegenkamp, Clark, Schoeller, & Landry, 2011) level of technical expertise (Hume &

Marfell-Jones, 2008; Ruiz, Colley, & Hamilton, 1971) and equipment calibration (Marfell-Jones, Stewart, & de Ridder, 2012). Biological variation may result from food and fluid ingestion or exercise prior to assessment and appears to influence most body composition methods albeit to different degrees (Bone et al., 2016; Kerr et al., 2017). Other biological variables known to impact on estimates of body composition include body temperature and skin moisture (Fields, Higgins, & Hunter, 2004), gastrointestinal contents (Bone et al., 2016) and muscle solutes (Rouillier, David-Riel, Brazeau, St-Pierre, & Karelis, 2015).

While quantification of precision error (PE) is frequently done by calculating differences in same-day repeat assessments of body composition (Hangartner, Warner, Braillon, Jankowski, & Shepherd, 2013; Hind et al., 2018) using least significant change (LSC) values, this fails to account for biological variability in the absence of controls, and can be evident during longitudinal monitoring (Meyer et al., 2013). Given this, we have advocated for identifying the LSC for same-day (technical error) and consecutive-day (biological variation) precision in estimates of FM and FFM, finding a more accurate interpretation of true and meaningful change (Zemski et al., 2019). Currently, the precision of BOD POD, BIS and SA using LSC values for same-day and consecutive-day analysis has not been explored. Therefore, aims of this study were to 1) to establish the same-day technical error of the four methods and 2) determine the consecutive-day PE of the methods using LSC values to determine the threshold of meaningful change in resistance trained male athletes.

Methods

Subjects

Thirty-two Caucasian volunteers participated in this study and met the inclusion criteria which included being male gender, at least two or more years resistance training experience, and with a BMI of ≥ 25 . Subjects were excluded from the study if they were >190 cm tall due to the limitation of the active scanning area of the DXA bed. Characteristics of all individuals are presented in Table 1. All subjects were informed of the nature and possible risks of the investigation before giving their written informed consent. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving subjects were approved by the Human Research Ethics Committee of the University of the Sunshine Coast (Ethics Approval No. S/12/450).

Experimental design

Each subject underwent three testing sessions during a 24-h window over a two day period (Fig. 1) with each measurement taken by the same technician. The sessions commenced with body mass and stretch stature measured in minimal clothing, a total body DXA scan immediately followed by a BIS estimation of total body water (TBW), a BOD POD test and an assessment of subcutaneous FM via the skinfold technique, in that sequence. Each subject undertook tests 1 (D1T1) and 2 (D1T2) on day 1 under standardised conditions (early morning, overnight fasted, well rested and bladder voided). D1T2 was undertaken immediately after D1T1 and test 3 (D2) was undertaken the following morning, 24 hours after D1T1. Comparison of these testing sessions allowed the calculation of typical error of measurement (TEM), random consecutive-day biological variability, and the difference in estimates of body composition data.

117 *Subject Presentation*

118

119 Guidance was provided on both days to encourage adherence to standardised presentation
120 for all three of the tests (D1T1, D1T2 and D2). Subjects were required to present overnight
121 fasted, bladder voided and well rested (no prior physical activity) on the mornings before
122 D1T1 and D2. They were asked to wear minimal fitted clothing with metal objects and
123 jewellery removed, plus clothing checked for metal zips or studs. Hydration status was
124 assessed by a mid-stream sample of urine provided by the subjects early on both mornings
125 before testing (D1T1 and D2). The specific gravity of the urine sample was measured using a
126 digital refractometer (UG-Alpha; Atago Corporation). All subjects voided their bladder
127 before tests.

128

129 *Dual-energy X-ray absorptiometry*

130 All DXA scans were undertaken in the total body mode on a pencil beam DXA scanner (Lunar
131 DPX; GE Healthcare) with analysis performed using GE enCORE version 13.60 software (GE
132 Healthcare) with the combined Geelong/Lunar reference database. CV for the laboratory
133 being 0.1, 2.2, 0.6 and 1.0% for BM, FM, lean mass and BMC respectively. The DXA was
134 calibrated with phantoms as per the manufacturer's guidelines each day before
135 measurements were taken. All scans were conducted by the same Queensland Radiation
136 Health licensed technician using the standard thickness mode as determined by the auto
137 scan feature in the software and all safety protocols as per the Institution's Radiation Safety
138 Protection Plan were adhered to. The scans were performed according to a protocol
139 developed that emphasises a consistent positioning of subjects on the DXA scanning bed
140 (Alisa Nana, Gary J Slater, Will G Hopkins, & Louise M Burke, 2012a) as previously described

(Alisa Nana, Gary J. Slater, Will G. Hopkins, & Louise M. Burke, 2012b). In addition, two Velcro straps were used to minimise any subject movement during the scan as well as provide a consistent body position for subsequent scans. One strap was secured around the ankles above the foot positioning pad and the other strap was secured around the trunk at the level of the mid forearms (Kerr et al., 2016). All scans were analysed automatically by the DXA software, but all regions of interest were reconfirmed before being included in the subsequent statistical analysis.

Bioelectrical impedance spectroscopy

Immediately after each DXA scan, whilst the subjects were still positioned on the DXA scanning bed, body composition derived from TBW obtained values, was measured using the SFB7 BIS device (ImpediMed, Brisbane, Australia). Subject positioning was standardised (Kyle et al., 2004) to ensure supine positioning on the non-conductive foam mattress without contact to the metal side supports of the DXA scanner for a minimum of 15 min before BIS measurements (Ward, Isenring, Dyer, Kagawa, & Essex, 2015). The BIS was calibrated as per the manufacturer's instructions with each participant's stature, body mass, age and sex programmed into the unit. Sites of attachment for the electrodes (ImpediMed) were first shaved and cleaned with alcohol wipes before the dual-tab electrodes were attached as follows: one electrode was attached centrally on the top side of the wrist in alignment with the ulnar head and 5 cm lower on the dorsal surface of the hand. The second electrode was attached centrally on the dorsal surface of the ankle between the lateral and medial malleoli and 5 cm lower on the dorsal surface of the foot which is in accordance with previous guidelines (Ava Kerr, Slater, Byrne, & Chaseling, 2015). The SFB7

measures impedance using 256 frequencies between 4 and 1024 kHz to estimate TBW based on a Cole-Cole plot (Cornish, Ward, Thomas, Jebb, & Elia, 1996). Three measurements were taken consecutively and the median of these used in subsequent analysis. The TBW value, as per the Pace et al model (Pace & Rathbun, 1945), was used to estimate body composition of FFM and FM by simple subtraction from body mass.

Air displacement plethysmography

Immediately after BIS measurement, assessment of body density was undertaken using the BOD POD (Life Measurement Instruments) following the recommended procedures of the manufacturer (Dempster & Aitkens, 1995) utilising a validated, predicted thoracic lung volume (VTG) estimation (McCrory, Molé, Gomez, Dewey, & Bernauer, 1998). Subjects wore Lycra clothing and a silicone swim cap, with all metal objects removed before measurement. Body density was calculated by the BOD POD's software system (COSMED version 5.3.2) as follows:

$$D \text{ (density)} \text{ Mass (scale)} = \text{Volume (BOD POD)}$$

An estimate of FM and FFM was obtained to calculate %BF as defined by the Siri equation (Siri, 1961), as follows:

$$\%BF = (497.1/\text{body density}) - 451.9.$$

Surface anthropometry

Immediately after completion of the BOD POD assessment, duplicate skinfold

measurements were taken according the International Society of the Advancement of Kinanthropometry (ISAK) technique by the same technician certified by ISAK as previously described (Norton et al., 1996).

The sum of eight skinfolds was determined following measurements of the triceps, biceps, sub scapulae, iliac crest, supra-spinal, abdominal, quadriceps and calf skinfold using a calibrated skinfold caliper (Harpender; Bate International). Due to the similar procedure, equipment and population used, the 4C validated Evans equation of three skinfolds (triceps, abdominal and thigh) was utilised to calculate %BF as (Evans, Rowe, Misic, Prior, & Arngrímsson, 2005):

$$\%BF = 8.997 + 0.24658 \times (3SKF) - 6.343 \times (gender) - 1.998 \times (race),$$

Gender coded as 0 = female, 1 = male, and race coded as 0 = white, 1 = black.

Stretch stature was measured with a stadiometer (Harpender; Holtain Limited) to the nearest 0.1 cm. Body mass was measured on a calibrated scale to the nearest 0.01 kg (SECA GMBH).

Statistical analysis

Data analysis was performed using Microsoft Excel (Microsoft, Redmond, WA). Descriptive data are reported as the mean \pm standard deviation (SD). The precision is reported as the root mean-square SD (RMS_ SD) and percentage coefficient of variation (%CV). The resulting LSC with 95% confidence intervals (LSC_ 95% CI) were calculated following the ISCD protocol (Hangartner et al., 2013). The %CV was derived from the equation $\%CV = (SD / \text{mean}) * 100$. Coefficients of determination (R^2) were calculated for measurements to establish the relationship between same-day and consecutive-day measures. Paired t tests were utilized to test for differences based on same-day versus consecutive-day test results and precision for all techniques. Statistical significance was set at 0.05. Bland Altman plots were created to compare individual same-day and consecutive-day precision for all techniques.

Results

Descriptive statistics for the participants in this study are given in Table 1. The mean differences between same-day (technical error) and consecutive-day (technical error and biological variation) for FM and FFM in all methods are shown in Table 2. Differences between same-day and consecutive-day testing demonstrating the LSC values for all methods are given in Figure 2.

Table 3 shows the PE for each method of testing, represented as the %CV, with the RMS-SD, LSC and %LSC. Strong agreement was found for all methods for same-day, and for consecutive-day FM regression analysis (SA $R^2 = 1.00 - 1.00$, BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 1.00 - 0.99$, BIS $R^2 = 0.98 - 0.96$) as shown in Figures 3 and 4. Regression analysis undertaken for same-day and consecutive-day FFM for all methods revealed strong relationships (SA $R^2 = 1.00 - 1.00$, BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 0.99 - 0.99$, BIS $R^2 =$

0.99 – 0.96) as shown in Figures 5 and 6.

Bland Altman analysis revealed SA had the smallest level of bias between same-day and consecutive-day precision for FM (83 g) and FFM (178 g) with very low limits of agreement (FM: -7 g to 173 g; FFM: -185 g to 172g). DXA and BOD POD had low levels of bias between same-day and consecutive-day precision for FM (DXA: 226 g; BOD POD: 318 g) and FFM (DXA: 309 g; BODPOD: 321 g) with low limits of agreement for DXA (FM: -365 g to 87 g; FFM: -59 g to 558 g) and for BOD POD (FM: -275 g to 361 g; FFM: -251 g to 390 g). The largest level of bias between same-day and consecutive-day precision came from BIS for FM (524 g) and FFM (580 g) with wider limits of agreement (FM: -108 to 939 g; FFM: -930 g to 230 g) as shown in Figures 7 and 8.

Discussion

To our knowledge this is the first study exploring both technical error and the short-term biological variation within a 24-hour period, using four independent methods of body composition assessment. The body composition PE was greater when quantified from consecutive-day compared to same-day results on a resistance trained athletic male cohort. This was evident across all body composition assessment techniques. Consecutive-day PE was 25% higher for DXA and BOD POD (FM and FFM) estimations and nearly 50% higher for BIS (FM and FFM) and SA (FFM) than same-day PE. It must be noted that same-day and consecutive-day PE in SA (FFM) was lower than all other methods. In contrast the SA FM PE for same-day and consecutive day analysis was lower for consecutive-day but only by 8% and was not significantly different ($p < 0.5$). This shows that biological variation affects measurement precision even within very short time frames (24 hours), at least when using

BIS, DXA and BOD POD methodology. Therefore, the use of consecutive-day PE is advocated as longitudinal monitoring of physique will always include both technical error and biological variation.

Excellent SA same-day precision was found for estimations of FM (CV 1.0%) and FFM (CV 0.2%) as well as for consecutive-day testing with FM (CV 1.0%) and FFM (CV 0.3%) respectively. Raw measurements from SA (mm) have been shown to be robust and unaffected by biological variation caused by prior food and fluid ingestion or exercise (Kerr et al., 2017) yet this study included body mass to obtain estimates of FM and FFM using the Evans equation (Evans et al., 2005). It would be expected then, that consecutive-day PE would be larger given that body mass is acutely influenced by hydration status, gastrointestinal tract contents and muscle glycogen stores (Rouillier et al., 2015). Due to adopting previous recommendations of subject presentation including overnight fasting, post bladder and bowel evacuation with body measurements taken early in the morning in minimal clothing, the biological impact on precision was expected to be minimal (Kerr et al., 2017; Nana, Slater, Stewart, & Burke, 2015).

DXA is prone to biological variance due to changes in hydration, significantly affecting FFM estimates (Kerr et al., 2017). This is particularly noticeable in large muscular males with high levels of FFM (Barlow et al., 2015; J. C. Bilsborough et al., 2014). Previous literature and manufacturing guidance suggest that a standardized testing protocol be adopted to minimise technical error and biological variation (Kerr et al., 2016; Nana et al., 2012a). This is in agreement with the literature finding a CV of 0.5 and 1.5% respectively (De Lorenzo, Andreoli, & Candeloro, 1997; Nana et al., 2012a) and more recently, results from Zemski et

al with a consecutive-day FM CV of 2.9% and lean mass CV of 1.1% (Zemski et al., 2019). Despite obtaining excellent precision from utilising a standardized presentation protocol those authors found biological variance (consecutive-day) to be higher than technical error (same-day) most probably due to short-term changes in hydration (Nana et al., 2012a), sleep hygiene (Vitale, Owens, Hopkins, & Malhotra, 2019) and intramuscular solute levels (Bone et al., 2016). These findings would support the results from this study with a FM and FFM CV of 2.4 and 0.5% respectively. While current best practice guidance was followed, this may not account for variance in muscle solute content which is known to influence reliability. The impact of standardised training and diet on consecutive-day precision warrants investigation.

Close comparisons between DXA and BODPOD were identified in this study with strong agreement found in same-day and consecutive-day FM PE (BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 1.00 - 0.99$) and FFM (BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 0.99 - 0.99$). In support, previous research using DXA and BODPOD technology shows consistent results with this study, with only small or trivial PE in FM and FFM estimates from consecutive-day testing conducted under standardised presentation conditions (Kerr et al., 2017). Despite BOD POD estimates of FM and FFM being subject to biological variation if unrestricted subject presentation occurs (food and fluid intake plus physical activity), BOD POD precision in this study showed that very high resolution can be obtained if these variables are controlled for (FM CV 2.8%, FFM CV 0.6%). A limitation of this study is that the DXA scanner used to estimate body composition (GE Lunar DPX Pro) has been superseded by newer models with enhanced precision. PE from the DPX estimations has been found to be twice as high as the GE Lunar Prodigy in athletes (J. C. Bilsborough et al., 2014) whereas the iDXA model

resolution has improved bone edge detection thus allowing superior algorithms for body composition estimation (Toombs, Ducher, Shepherd, & De Souza, 2012).

Factors that impact TBW such as prior food and fluid intake, physical activity or medical conditions make BIS vulnerable to imprecision (Kyle et al., 2004). Additionally, variance in fluid and electrolyte content will affect TBW (Saunders, Blevins, & Broeder, 1998) and confound any change in physique traits inferred from BIS (O'brien, Young, & Sawka, 2002). Given normal daily fluctuations in TBW, it is unsurprising that the change between same-day and consecutive-day precision using BIS derived estimates of FM and FFM showed nearly a 50% increase in PE for both FM (3607 vs 2331 g) and FFM (3966 g vs 2276 g) estimates. BIS also had the highest CV % of all methods for both same-day FM and FFM (5.2% and 0.6%) and consecutive-day values (9.4% and 1.1%) respectively. This suggests that despite implementing a rigorous athlete presentation protocol prior to testing, a lower tolerance level for precision still occurs. Given this, the ability of BIS to accurately track small changes in physique among athletic populations is questionable.

Conclusion

In conclusion, consecutive-day PE was larger than same-day for FM and FFM estimates obtained from DXA, BOD POD, and BIS (except for SA FM which was marginally lower) in a cohort of muscular resistance trained male athletes. This is despite PE limits for FM and FFM estimates being within acceptable precision thresholds, at least for DXA. Clearly all methods are subject to some imprecision due to daily biological fluctuations, especially BIS which calculates physique traits from a TBW estimation. Given that both technical error and biological variation contribute to precision, we recommend the use of LSC values calculated

from consecutive-day analysis when interpreting longitudinal change for true changes in physique. Application of DXA, BOD POD or SA should be advocated over BIS for athletic populations where only small changes are observed over time.

Practical Implications

Adopting LSC values from consecutive-day analysis likely provide a more appropriate benchmark to assess meaningful change in body composition of athletic populations longitudinally.

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Figure 1 Study design of three testing sessions conducted over 24 hours.

Figure 2 Least significant change for DXA, BODPOD, BIS and SA for same-day and consecutive-day measures.

Figure 3 Regression analysis between measures of fat mass (FM) for same-day precision.

Figure 4 Regression analysis between measures of fat mass (FM) for consecutive-day precision.

Figure 5 Regression analysis between measures of fat free mass (FFM) for same-day precision.

Figure 6 Regression analysis between measures of fat free mass (FFM) for consecutive-day precision.

Figure 7 Bland Altman plots for differences in same-day vs consecutive-day measures for fat mass (FM).

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521 **Figure 8** Bland Altman plots for differences in same-day vs consecutive-day measures for fat
522 free mass (FFM).